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IN THE CLAIMS:

Claims 1-14 (Canceled)

15. (Currently Amended) A composition ~~for immunizing an animal against bacterial infection~~ comprising:

a pharmaceutically acceptable carrier, diluent or excipient; and at least one non-virulent strain of bacteria produced by the process comprising:

5 introducing at least one mutation into the genome of a bacteria;

culturing the mutated bacteria in the presence of an antimicrobial agent that kills growing but not non-growing bacteria;

selecting surviving bacteria;

10 testing the selected surviving bacteria for virulence;

and selecting the non-virulent strains.

16. (Original) The composition of claim 15, wherein said bacteria is a mycobacteria.

17. (Original) The composition of claim 16, wherein said bacteria is a slow growing mycobacteria.

18. (Original) The composition of claim 17, wherein said slow growing mycobacteria is *Mycobacterium paratuberculosis*.

19. (Original) The composition of claim 15, wherein said mutation is by insertion of a transposon.

20. (Original) The composition of claim 15, wherein said mutation is a random mutation.

21. (Original) The composition of claim 15, wherein said antimicrobial agent is a fluoroquinolone.

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22. (Original) The composition of claim 21, wherein said fluoroquinolone is Bay y 3118.

23. (Previously Amended) The composition of claim 22, wherein said Bay y 3118 is used at a concentration between of at least 0.015 µg/mL.

24. (Original) The composition of claim 15, wherein said antimicrobial is D-cycloserine.

25. (Original) The composition of claim 24, wherein D-cycloserine is used at a concentration of at least 25 µg/mL.

26. (Original) The composition of claim 15, wherein said mutated bacteria is cultured in an intracellular culture system.

27. (Original) The composition of claim 26, wherein said intracellular culture system is a macrophage culture system.

28. (Currently Amended) A composition ~~for immunizing an animal against *Mycobacterium paratuberculosis*~~ comprising:

a pharmaceutically acceptable carrier, diluent or excipient;

and at least one non-virulent strain of *M. paratuberculosis* produced by the

process comprising:

introducing at least one random mutation into the genome of a strain of *M.*

paratuberculosis by insertion of a transposon;

infecting macrophages with the mutated strain;

culturing the infected macrophages in the presence of a fluoroquinolone or D-

cycloserine;

selecting surviving *M. paratuberculosis* organisms;

testing the selected surviving organisms for virulence in an animal; and

selecting the non-virulent strains.

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29. (Currently Amended) A composition for immunizing an animal against a bacteria comprising:

a pharmaceutically acceptable carrier diluent or excipient;

and at least one bacterial virulence determinant, the determinant identified by a

5 process comprising;

introducing at least one mutation into the genome of a bacteria;

culturing the mutated bacteria in the presence of an antimicrobial agent that kills growing but not non-growing bacteria;

selecting surviving bacteria;

10 testing the selected surviving bacteria for virulence;

selecting the non-virulent strains;

sequencing genetic material from the selected non-virulent bacteria to determine the site of the mutation; and

identifying the virulence determinant based on the site of the mutation.

30. (Original) The composition of claim 29, wherein said bacteria is a mycobacteria.

31. (Original) The composition of claim 30, wherein said mycobacteria is a slow growing mycobacteria.

32. (Original) The composition of claim 31, wherein said slow growing mycobacteria is *Mycobacterium paratuberculosis*.

33. (Original) The composition of claim 29, wherein said mutation is by insertion of a transposon.

34. (Original) The composition of claim 29, wherein said mutation is a random mutation.

35. (Original) The composition of claim 29, wherein said antimicrobial agent is a fluoroquinolone.

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36. (Original) The composition of claim 35, wherein said fluoroquinolone is Bay y 3118.

37. (Original) The composition of claim 36, wherein said Bay y 3118 is used at a concentration of at least 0.015 µg/mL.

38. (Original) The composition of claim 29, wherein the antimicrobial is D-cycloserine

39. (Original) The composition of claim 38, wherein said D-cycloserine is used at a concentration of at least 25 µg/mL.

40. (Original) The composition of claim 29, wherein said mutated bacteria is cultured in an intracellular culture system.

41. (Original) The composition of claim 40, wherein said intracellular culture system is a macrophage culture system.

42. (Currently Amended) A composition for immunizing an animal against *Mycobacterium paratuberculosis* comprising:

a pharmaceutically acceptable carrier diluent or excipient;

and at least one *Mycobacterium paratuberculosis* virulence determinant, the determinant identified by a process comprising;

introducing at least one mutation into the genome of a strain of *Mycobacterium paratuberculosis* by insertion of a transposon;

infecting macrophages with the mutated strain;

culturing the infected macrophages in the presence of a fluoroquinolone or D-cycloserine;

selecting surviving bacteria;

testing the selected surviving bacteria for virulence in an animal;

selecting the non-virulent bacteria;

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- 15 sequencing genetic material from the selected non-virulent bacteria to determine
the site of the mutation; and
determining the virulence determinant based on the site of the mutation.

Claims 43-53 (Canceled)